

tem Science Co., Ltd., SEQ ID NO: 2, see **135** in FIG. **14C** and FIG. **15C**] and 1 μ M of AlexaFluor750-labeled DNA [manufactured by Japan Bio Services Co., LTD., SEQ ID NO: 3, see **137** in FIG. **14D** and FIG. **15C**] were incubated in a hybridization solution [trade name: PerfectHyb, manufactured by TOYOBO CO., LTD] at 60° C. for 1 hour to obtain a labeled form [see **130b** in FIG. **15C**]. The labeled form **130b** is composed of an AlexaFluor750-labeled DNA **137** composed of a labeling substance **133** and a DNA **136** and a labeling substance-retaining DNA **135**.

(1-3) Trapping of Analyte

[0187] Silicone rubber (0.1 mm in thickness) was placed around the working electrode **61** of the working electrode substrate (the upper substrate **30**) obtained in a similar manner to (1-3) of Test example 1-1 so that a partition was formed. CK19 DNA-trapping DNA (trapping substance **81**) [see FIG. **14E**, SEQ ID NO: 4] was immobilized on the working electrode body **61** of the working electrode substrate (the upper substrate **30**).

[0188] Thereafter, a hybridization buffer [trade name: Perfect Hyb, manufactured by TOYOBO CO., LTD] and 1 nM of CK19 DNA [150 bases, SEQ ID NO: 5, Manufactured by Hokkaido System Science Co., Ltd., see FIG. **14F**] as the analyte S were placed in the space surrounded by the working electrode substrate (the upper substrate **30**) and the silicone rubber. The working electrode substrate (the upper substrate **30**) was incubated at 60° C. for 2 hours. Thus, the analyte S was trapped by the trapping substance **81** on the working electrode body **61** [see FIG. **15A**].

[0189] After discharge of the reaction solution, a hybridization buffer [trade name: Perfect Hyb, manufactured by TOYOBO CO., LTD.] and 50 nM DNA binding BSA (conjugate) [see **130a** in FIG. **15B**] were placed in the above space. The working electrode substrate (the upper substrate **30**) was incubated at 60° C. for 1 hour. Thus, the analyte S and the DNA binding BSA **130a** (conjugate) were trapped on the working electrode body **61** [see FIG. **15B**] by hybridizing the analyte S trapped by the trapping substance **81** on the working electrode body **61** with a CK19-recognizing DNA **132** (the first binding substance) included in a DNA binding BSA **130a** (conjugate).

[0190] After discharge of the reaction solution, a hybridization buffer [trade name: Perfect Hyb, manufactured by TOYOBO CO., LTD.] and the labeled form **130b** were placed in the above space. The labeling substance-retaining DNA **135** in the labeled form **130b** (the second binding substance) was hybridized with the label-retaining DNA-binding DNA (the first linker) [see **134** in FIG. **15B**] included in the DNA binding BSA **130a** (conjugate) immobilized on the working electrode body **61** via the trapping substance **81** and the analyte S by incubating the working electrode substrate (the upper substrate **30**) at 60° C. for 1 hour. Thus, a complex containing the trapping substance **81**, the analyte S, the DNA binding BSA **130a** (conjugate), and the labeled form **130b** was formed on the working electrode body **61** of the working electrode substrate (the upper substrate **30**) [see FIG. **15C**] (Example 1-2). A label-retaining DNA-binding DNA **134** (the first linker) is bound to the 3' terminal region [the italicized sequence in FIG. **14C**] of the labeling substance-retaining DNA **135** (the second binding substance).

(2) Control Experiment

[0191] The same operation as Example 1-1 was performed except that 1 μ M of CK19 recognizing/label-retaining DNA

[SEQ ID NO: 6, see FIGS. **14G** and **140a** in FIG. **16**] was used in place of the DNA binding BSA in Example 1-2. Then, a complex containing the trapping substance **81**, the analyte S, a CK19 recognizing/label-retaining DNA **140a**, and the labeled form **130b** was formed on the working electrode body **61** of the working electrode substrate (the upper substrate **30**) [see FIG. **16C**] (Comparative example 1-2).

(3) Measurement of Photocurrent

[0192] Silicone rubber was placed around the working electrode substrates of Example 1-2 and Comparative example 1-2 so that a 0.2-mm-thick side wall was formed. Then, the space surrounded by the working electrode substrate (the upper substrate **30**) and the silicone rubber was filled with the electrolytic solution obtained in Preparation example 1-2. The space filled with the electrolytic solution was sealed with the counter electrode substrate obtained in Preparation example 1-3 from the upper side of the working electrode substrate. Thus, the working electrode and the counter electrode are brought into contact with the electrolytic solution. Then, the detection chip including the working electrode substrate and the counter electrode was placed in an electrochemical measurement device. The working electrode lead and the counter electrode lead were connected to the ammeter.

[0193] The light source (wavelength: 781 nm, laser light source with an output power of 13 mW) emits light from the working electrode substrate side toward the counter electrode substrate. The labeling substance is excited by photoirradiation, thereby generating electrons. When the generated electrons are transported to the working electrode, current flows between the working electrode and the counter electrode. Then, the electric current was measured. FIG. **17** shows examined results of a relationship between the kind of the detection method and photocurrent in Test example 1-2.

[0194] From the results shown in FIG. **17**, it is found that the current detected in Example 1-2 in which BSA (a support composed of polypeptide) is used as the support retaining the labeling substance is about 218.2 nA. On the other hand, it is found that the current detected in Comparative example 1-2 in which the support composed of polypeptide is not used as the support retaining the labeling substance like a conventional manner is about 5.8 nA. From these results, it is found that a very large current can be detected when the support composed of polypeptide is used as the support retaining the labeling substance.

Test Example 1-3

(1-1) Production of DNA Binding BSA

[0195] BSA (manufactured by Sigma) was purified by gel filtration chromatography. The purified BSA [see **151** in FIG. **19B**] and AlexaFluor750 derivative (trade name: AlexaFluor750 carboxylic acid, succinimidyl ester, manufactured by Invitrogen) were reacted in 0.1 M sodium carbonate (pH 8.5) at room temperature for 1 hour. The obtained reaction product was filtered through a desalting column [trade name: Zeba Spin Micro desalting Column, manufactured by Pierce] to remove the unreacted AlexaFluor750 derivative, and AlexaFluor750-labeled BSA was obtained. The AlexaFluor750-labeled BSA was assumed to have a structure in which about ten AlexaFluor750 derivatives were bound to BSA based on the band shift and absorption spectrum by SDS-PAGE. The complex was reacted with a cross-linker [trade name: GMBS,